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The Chromatophores of *Fundulus heteroclitus* in Polarized Light.

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(Plates I–III).

Introduction.

It has been shown by several investigators that certain tissues and many cytological structures having a high degree of organization will show the phenomenon of birefringence (see Schmitt, 1940, and Fischer, 1941). The possibility that chromatophores may be a modified form of muscle as indicated by Spaeth (1916) suggested the use of polarized light for this study. By this means we have found doubly refracting material to be definitely associated with pigment cells on the scales of *Fundulus heteroclitus*. Our observations on this phenomenon are the subject of this paper.

Procedure.

The instrument employed was a Bausch and Lomb microscope fitted with apochromatic lenses. To this the Nichols prisms from a polarimeter were added. The polarizer was mounted below the substage and the analyzer above a high point ocular. The illumination was provided by a 100 watt G. E. projection lamp giving an intense point source of light. Birefringence was detected in the usual manner, that is, by the brilliance of the material when the prisms are set with their planes of polarization at right angles, in which condition the rest of the field is dark. The intensity of the light from the birefringent material was taken as an indication of the degree of double refraction.

All observations were made on the pigment cells of scales taken from the dorsal and dorsolateral surface of *F. heteroclitus*. The scales were mounted as hanging drop preparations or on ordinary flat slides with coverslips; the latter method permitted the introduction of different solutions as well as the study of the effect of pressure while the objects were still in focus.

Observations.

The appearance of chromatophores of a scale under low power in polarized light is shown in Plate I. The widespread distribution of doubly refracting material is at once apparent. Plate II shows two birefringent bodies highly magnified; the one in sharp focus clearly reveals delicate strands coming off the processes. In Plate III the same area as II is shown in ordinary transmitted light; here the birefringent masses can be seen faintly.

Examination of these objects with reflected light reveals them to be the guanophores described by Odiorne (1933). Our observations indicate that some of the centrally located iridocytes of the "melaniridosomes" described by Foster (1933, 1937) and shown in a photograph by Odiorne (1933) are the same doubly refracting material described in this paper in a highly birefringent state. We have found, too, that the doubly refracting substance shows a distinct relationship among melanophores and between xanthophores and melanophores as well. For example, under proper conditions each melanophore on a scale, whether dispersed or condensed, reveals birefringent material which sends out strands interconnecting with the birefringent material of other melanophores as well as with similar material associated with xanthophores. By careful focusing under oil, the xanthophores definitely are seen to lie within the substance showing birefringence; the same may be true for the melanophores although similar observations are prevented by the dense melanin granules.

From the start of our studies on birefringence it became obvious that this property could vary from zero to a certain maximum. Thus, scales, isolated from a fish kept in a refrigerator for several days, showed little or no sign of this condition when first observed under the microscope; within a few moments a decided network became apparent, many of its strands arising from the tips of the larger birefringent processes in the manner shown in Plate II. Closer observation revealed that the "contracted" melanophores were slowly "expanding" and that at least some of their processes containing the melanin were directed along the birefringent strands. Both the melanin processes and the birefringent strands in these cases were at the same focal level as well as similarly oriented, and no sharp line of demarcation was apparent between them. This relationship was further confirmed when pressure on the coverslip produced a succession of waves which arose at the base of a process containing melanin and continued outward along a birefringent strand. The transmission of these waves indicated two things more: (1) The birefringent and melanophore processes can have a certain rigidity and flexibility of structure and possibly a certain degree of contractility; (2) the branching network is not an illusion, for these waves can be seen to pass on to the rest of the birefringent network. The elastic return of displaced melanin granules observed by Behre (1935) confirms the first conclusion.

It was soon noticed that the magnitude of the birefringence could be intermediate to the extremes which were first observed. In this intermediate state the birefringent material showed a decided Brownian movement. Spontaneous changes in the degree of birefringence could also be seen. By watching granules obviously fixed in the surface of a birefringent process showing Brownian movement, it could be seen that their relative positions were unaltered even at the tip while this process was retracted. This would suggest a movement more to be expected of muscle fibers than of pseudopodia. The rigidity of this surface was also revealed when pressure of the coverslip caused a small, highly birefringent mass to move through one of the birefringent processes as though through a channel; this mass was followed by a cluster of melanin granules, which further indicates *the close association of melanophores and these doubly refracting bodies.*

It has been possible to watch other individual particles from birefringent strands which, like melanin granules, may apparently be left behind as the doubly refracting processes retract. These particles themselves are birefringent and appear to rotate, for this birefringence alternately appears and disappears with great regularity. Eventually, this apparent rotation ceases. Such behavior was demonstrated when an unusually thick connecting strand with Brownian movement gradually became thin midway until nothing but a fine thread with these twinkling granules was evident; this thread soon disappeared from sight but the granules remained visible. The implication would be that such connecting strands may be present though undetectable even in polarized light.

The effects of adrenalin chloride (1 : 1000 and 1 : 2000 in sea water diluted to 0.1 normal) and 5% ether were examined. The former led to the disappearance of Brownian movement in the birefringent masses and to an increase in the intensity of birefringence, while the latter reduced the birefringence to the point of extinction, often without the return of Brownian movement. Both produced their effects in a matter of minutes. The action of ether could not be reversed with adrenalin. Ninety-five per cent alcohol does not cause the disappearance of birefringence intensified by adrenalin, although the less intense birefringence associated with Brownian movement may disappear. Tenth normal KCl, like adrenalin, leads to increased birefringence accompanied by the usual disappearance of Brownian movement. Return to tenth normal sea water reverses this. The action of these substances parallels their effect on melanophores; KCl and adrenalin cause the "contraction" of melanophores accompanied by a decrease in Brownian movement (Spaeth, 1916) and 5% ether has the opposite effect (Wyman, 1924).

According to Odiorne (1933), when melanophores are "expanded" the guanophores are "contracted" and invisible. However, we have found that animals kept continuously on a dark background show a relative increase in the amount of birefringent material associated with the melanophores. This suggests a similarity of behavior in both melanophores and "guanophores." The identity of the effects of adrenalin and KCl with reference to Brownian movement in both structures further corroborates this conclusion.

Discussion.

The presence of the birefringent material, its relationship to the chromatophores, and its behavior strongly suggest that this doubly refracting material is an integral part of chromatophores, at least for the melanophores and xanthophores on the scales of *Fundulus heteroclitus.*

Apparently we are dealing with a material which can exist in various states of solvation and molecular orientation. Complete lack of birefringence indicates a minimum of organization and a maximum of solvation while strong birefringence argues for a minimum of solvation and a maximum of orientation. Such changes are now well-known for chromosomes (Schmitt, 1940). Similar changes are attributed to muscle (Fischer, 1941).

The failure of birefringence to disappear in concentrated alcohol decreases the likelihood that lipoids are involved and strengthens the possibility that the molecules concerned are proteins as in muscle. It might be argued that orientation of the contained birefringent particles (believed by

Odiorne to be guanin) is concerned in the changes of birefringence. However, it is difficult to see how one could completely assign the present observations to changes in the orientation of these granules, especially in view of the complete disappearance of birefringence in 5% ether although the granules remain visible and in view of the ease with which the particles can be distinguished within the surface of the doubly refracting processes. Certainly the crystallizing out of guanin is not involved since in the strongly birefringent state no sign of the structure typical of guanin crystals is apparent.

If the movements of the pigment and other granules are associated with the changes in birefringence, as our observations tend to indicate, the following interpretation is possible. The various constituents of the chromatophores, such as xanthin and melanin, can form part of the structural pattern of the chromatophores. The degree of development of the pattern itself is governed by the metabolic state of the protoplasm; such substances as adrenalin, KCl, ether, etc., would lead to changes in the organization by affecting the metabolic processes. Melanin and xanthin, if associated with phosphate, carboxyl, amine, or hydroxyl groups by being part of certain complexes, would be able to combine reversibly with similar groups of the protein pattern of chromatophores. Reversible combinations of this kind can account for the observed changes in the freedom of movement of these granules. As part of collapsing or expanding chains of molecules, the granules could participate in the changes which have been described as "contraction" and "expansion" of chromatophores. This concept is in accord with the elastic return of dispaced melanin granules observed by Behre (1935).

Gilson (1926) suggested that fine sheaths, which determine the shape of expanded melanophores, may extend into the tissue spaces. We have already pointed out that birefringent strands may appear and disappear without any evidence of movement of the particles within them, showing that a change in structure rather than a change associated with movement is involved. This would confirm Gilson's suggestion of preformed processes, but whether such processes are collapsed cylinders remains to be determined.

We have noted that the interconnecting strands among the chromatophores may be present all of the time and merely come into view under certain conditions. But this does not eliminate the possibility that part of the network may be formed by the outgrowth and fusion of new processes. Such behavior would be consistent with that which has been described for vertebrate smooth muscle: "Even in the adult vertebrate it is believed that smooth muscle cells may be derived from undifferentiated mesenchyme cells in connective tissues. The myoblasts become more and more elongate with development and appear still connected laterally by cytoplasmic processes" (Scott & Kendall, 1935). Furthermore, such outgrowth would provide an explanation for the migration of pigment cells beyond the boundaries of grafts demonstrated by DuShane (1935). Possibly the peripheral changes of denervated caudal bands which Parker (1936) has ascribed to "lipohumors" may also be accounted for in this way, especially since we have observed an increase in the extent and connections of birefringent material among chromatophores upon prolonged exposure to a dark background.

The staining techniques which have been employed to reveal the "nerves" running to chromatophores may, at least in part, have actually shown the birefringent strands; by virtue of their structure, these strands might be expected to coagulate into coarser fibrils as in the case of nerve axoplasm. Smooth muscle cells are also connected to each other by a substance showing a reticulum of fine fibers by silver techniques (Scott & Kendall, 1935).

Summary.

1. Chromatophores on the scales of *Fundulus heteroclitus*, when examined in polarized light, are found to be intimately associated with a birefringent material.

2. This birefringent material has processes which send out strands that interconnect the chromatophores.

3. The intensity of birefringence of both processes and strands varies according to the conditions to which the fish and isolated scales have been subjected.

4. Changes in birefringence are accompanied by the appearance and disappearance of Brownian movement; the latter is more pronounced when the magnitude of birefringence is intermediate to zero and a certain maximum.

5. The strands of birefringent material appear and disappear spontaneously without any apparent movement; in addition to such changes, the larger processes also show an ability to move.

6. When visible, the birefringent strands may reveal a certain degree of rigidity and even contractility.

7. The movement of melanin granules in the melanophores appears to be intimately associated with this doubly refracting material.

8. The action of adrenalin, ether, and KCl on the birefringent material is similar to that described by various investigators for the melanophore itself.

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EXPLANATION OF THE PLATES.

Plate I.

Chromatophores from the scale of *Fundulus heteroclitus* in polarized light. L. P.

Plate II.

Birefringent mass associated with melanophores. Note fine strands arising from the processes. H. P.

Plate III.

The same area as in Plate II in ordinary transmitted light. Note the faintly visible birefringent material. H. P.